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Subcellular Localization of the Autoimmune Regulator Protein

CHARACTERIZATION OF NUCLEAR TARGETING AND TRANSCRIPTIONAL ACTIVATION DOMAIN*

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Jukka Pitkänen‡, Perttu Vähämurto, Kai Krohn, and Pärt Peterson

From the Institute of Medical Technology, FIN-33014 University of Tampere and Department of Pathology, Tampere University Hospital, Tampere, Finland

The autoimmune regulator (*AIRE*) gene, defective in the hereditary autoimmune disease APECED, encodes a transcriptional regulator protein. AIRE is expressed in the medullary epithelial cells and monocyte-dendritic cells of the thymus with lower expression in the spleen, fetal liver, and lymph nodes. At the cellular level, AIRE is located in microtubular structures of the cytoskeleton and in discrete nuclear dots resembling ND10 nuclear bodies. We studied the determinants of the targeting of AIRE into these structures. We report here that the N-terminal HSR domain confers localization to the microtubular network whereas the C-terminal region contains a second nuclear localization signal. We also demonstrate that the consensus nuclear localization signal of AIRE is functional and that the HSR domain harbors a nuclear export signal. Accordingly, the nuclear export inhibitor leptomycin B partially inhibits the nuclear export of AIRE. From a functional standpoint, we show that AIRE can activate the interferon β minimal promoter in a transfection assay and demonstrate that the transcriptional activating function of AIRE is mediated by its two plant homeodomain (PHD) zinc fingers.

The gene defective in the disease autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED),[1] the autoimmune regulator (*AIRE*), encodes a protein of 57.5 kDa (1, 2). The predicted protein domains of AIRE suggest a role in transcriptional regulation: a conserved nuclear localization signal, two PHD-type zinc fingers, four LXXLL or nuclear receptor interaction motifs, and the SAND and HSR domains (1–3). In agreement, we and others reported recently that AIRE can activate transcription from a reporter gene when fused to a heterologous DNA binding domain (4, 5) and that it interacts with the co-activator CREB-binding protein (CBP) (4). We also showed that the HSR domain, similar to Sp100, mediates AIRE-AIRE homodimerization and that it is needed for the activating function (4).

The disease caused by the defects in AIRE, or APECED, is a rare autosomal recessive autoimmune disease characterized by defective tolerance to certain self-antigens (1, 2, 6). Based on its monogenic etiology, APECED can be thought of as a model of organ-specific autoimmune diseases and thus can provide insights into the pathogenesis of autoimmunity.

The expression of AIRE in human tissues is found mostly in the thymus, spleen, fetal liver, and lymph nodes (1, 2, 7, 8). In the thymus, AIRE is seen in two types of antigen-presenting cells: medullary epithelial cells and cells of monocyte-dendritic cell lineage that are central in the negative selection of self-reactive T cells (7). The subcellular localization of AIRE consists of several distinct patterns. In the cytoplasm of transiently transfected cells, AIRE forms a pattern resembling intermediate filaments or microtubules (7, 9, 10). Accordingly, colocalization with vimentin has been reported (9, 10). In the nucleus, AIRE forms discrete nuclear dots, bearing similarity to PML nuclear bodies (7, 9, 10). The nuclear dot pattern has also been demonstrated in human tissue sections (7).

In view of data on the subcellular localization of AIRE, we have determined the protein domains responsible for its targeting to specific cellular locations. We have also further studied the function of AIRE on a minimal promoter in a transfection assay and determined the activation domain. Finally, we have demonstrated a potential nuclear export signal in the N-terminal HSR domain of AIRE.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfections, and Immunofluorescence—U937 and COS-1 cells were maintained as monolayers in Dulbecco's modified Eagle's medium supplemented with 100 units/ml penicillin-streptomycin and 10% bovine calf serum (Life Technologies, Inc.).

The transfections were performed using calcium phosphate precipitation for COS-1 cells (11) or electroporation for U937 cells. For COS-1 transfections, 5×10^5 cells were grown on coverslips in 6-well plates and transfected with 1 μg of the appropriate expression vector. For U937 transfections, 1×10^6 cells were transfected with 1 μg of vector DNA. Immunofluorescence staining was performed 24 h post-transfection as described using a monoclonal anti-AIRE antibody (7).

In nuclear export studies, leptomycin B (LMB; from M. Yoshida, University of Tokyo) was added 24 h post-transfection initially at 5, 25, or 30 ng/ml, the cells were grown for 3 or 24 h and stained as above. In subsequent experiments, 10 ng/ml LMB was used. The proportion of cells showing either nuclear, combined nuclear and cytoplasmic, and just cytoplasmic staining was calculated.

Immunofluorescence data were acquired using an Olympus BX50 microscope. Images were captured with a Photometrics Imagepoint Cooled CCD video camera and IPLab Spectrum 3.1a software for the Macintosh.

Plasmid Constructs—Wild-type AIRE cDNA was cloned into the pSI mammalian expression vector (Promega) by polymerase chain reaction using primers with *Eco*RI and *Sal*I restriction sites in the 5′- and 3′-ends, respectively. The truncated AIRE fragments AIRE-(1–256), -(1–293), -(1–348), -(1–207), -(292–545), -(175–545), and -(84–545) and the missense mutation-containing cDNAs AIRE L28P, AIRE L28P/

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‡ To whom correspondence should be addressed: Inst. of Medical Technology, University of Tampere. Lenkkeilijänkatu 6, Fin-33101 Tampere, Finland. Tel.: 358-3-2158032; Fax: 358-3-2157332; E-mail: jp58218@uta.fi.

[1] The abbreviations used are: APECED, autoimmune polyendocrinopathy candidiasis ectodermal dystrophy; AIRE, autoimmune regulator; PHD, plant homeodomain; CREB, cyclic AMP response element-binding protein; LMB, leptomycin B; IFNβ, interferon β; GFP, green fluorescent protein; PML, promyelocytic leukemia; HSR, homogenously staining region; GST, glutathione *S*-transferase; NLS, nuclear localization signal; SAND, Sp100, AIRE, NucP41/75, and DEAF-1/suppressin.

TABLE I
Subcellular localization of various AIRE constructs
Cyto, cytoplasmic localization; nuc, nuclear localization; dot, nuclear dot staining; fibr, microtubular cytoplasmic staining are shown.

Construct	Localization			
	cyto	nuc	dot	fibr
1–545 (wild type)	+	+	+	+
1–256[a]	+	+	–	+
1–293	+	+	–	+
1–348	+	+	–	+
1–207	+	+	–	+
84–545	+[b]	+	–	–
175–545	+	+	–	–
292–545	+	+	–	–
L28P[a]	+	+	–	–
L28P/K83E	+	+	–	–
K83E[a]	+	+	–	+
C302P	+	+	+	+
C437P	+	+	+	+
C302P/C437P	+	+	+	+

[a] Mutations found in APECED patients (R257*X*, L28P, and K83E).
[b] AIRE-(84–545) was almost exclusively located in the nucleus.

K83E, and AIRE C437P were subcloned into pSI from various constructs described earlier (4) using standard techniques. The mutation C302P was engineered into the pSI-AIRE (wild type) vector to disrupt the first PHD zinc finger using the GeneEditor site-directed mutagenesis system (Promega). The patient mutation K83E was similarly engineered into wild-type pSI-AIRE. To create pSI-AIRE C302P/C437P, an *Eco*RI-*Sac*I digest from pSI-AIRE C302P was cloned into pSI-AIRE C437P. For pGFP-AIRE NLS the consensus nuclear localization signal plus six flanking amino acids on either terminus (amino acids 101–141) were cloned by polymerase chain reaction into pEGFP-C3 (CLONTECH). The different AIRE plasmid constructs used in the experiments are summarized in Fig. 1.

Protein Interaction Assays—Expression and purification of GST fusion proteins from GST-AIRE C302P and GST-AIRE C437P, *in vitro* translation from pSI-AIRE C302P and pSI-AIRE C437P, and GST pull-down assays were performed as described (4).

Promoter Assays—In assays that look at the effect of AIRE on the interferon β (*IFNβ*) minimal promoter, the reporter plasmid pIFNβ-LUC (from Kalle Saksela, University of Tampere) was used, which contains the *IFNβ* minimal promoter (nucleotides −55 to +19) upstream of the luciferase gene. The assays were performed as follows. 2 μg of the reporter was cotransfected with 2 μg of the appropriate pSI-AIRE construct by electroporation into 1×10^6 COS-1 cells. The cells were harvested and lysed 48 h after transfection, and luciferase activity was measured using the luciferase assay system (Promega). The luciferase values were normalized with respect to total protein concentration. All experiments were confirmed twice.

RESULTS

The N-terminal HSR Domain of AIRE Is Required for Tubular Localization—Earlier reports by us and others have found wild-type AIRE to be partially localized in tubular structures in the cytoplasm of transfected cells (7, 9, 10). To identify the domain in AIRE responsible for this particular localization, we performed transfections with several AIRE deletion constructs (Table I, Fig. 1).

Only AIRE fragments containing an intact N terminus formed the tubular cytoplasmic staining seen with wild-type AIRE. The required minimal domain probably lies in the HSR domain, within at least the first 207 amino acids because none of the N-terminal deletions, AIRE-(84–545), -(175–545), or -(292–545), exhibited a tubular pattern but were diffusely localized in the cytoplasm, whereas AIRE-(1–207) localized effectively into tubular structures (Table I). To further confirm this, we tested the missense mutation AIRE L28P, found in APECED patients, and a double mutant construct, AIRE L28P/K83E. We found that both constructs lacked the tubular cytoplasmic pattern. However, the patient mutant construct AIRE K83E showed a cytoplasmic tubular pattern similar to wild type (Table I and Fig. 2). The results were essentially the same in COS-1 and U937 cells. Thus, the N-terminal HSR domain of AIRE is required for tubular localization.

The Nuclear Localization Signal of AIRE Is Functional—Upon studying nuclear localization we observed that C-terminal deletion constructs lacking both (AIRE-(1–256), -(1–207), and -(1–293)) or one (AIRE-(1–348)) PHD zinc finger but containing the nuclear localization signal also localized in the nucleus, suggesting that the nuclear localization signal is functional. The proportion of cells showing nuclear localization was comparable with wild-type AIRE with AIRE-(1–207) and AIRE-(1–256) but was smaller with AIRE-(1–293) and AIRE-(1–348) (not shown).

The presence of a consensus nuclear localization signal in AIRE has been reported based on the amino acid sequence (1, 2). It has not, however, been shown to be functional. To address this question, a short fragment containing the AIRE NLS (amino acids 101–141) was cloned downstream of the green fluorescent protein (pEGFP-C3, CLONTECH) and transiently expressed in COS-1 cells. We found that the minimal nuclear localization signal effectively transported GFP into the nucleus (Fig. 3).

The C-terminal Region Is Involved in Nuclear Transport—Recently, we and others reported that AIRE can function as a transcriptional activator and that the PHD zinc fingers are important for that function (4, 5). We set out to determine the role of the PHD zinc finger-containing C terminus in the subcellular localization of AIRE.

N-terminal AIRE deletion constructs lacking the nuclear localization signal (AIRE-(175–545) and AIRE-(292–545)) were still transported into the nucleus, approximately as efficiently as wild-type AIRE (Table I). To investigate the role of the PHD zinc fingers in this nuclear transport we tested the missense mutants AIRE C302P and AIRE C437P. Both mutants, however, were effectively transported into the nucleus in a pattern indistinguishable from wild type (Table I). This would seem to indicate that the PHD zinc fingers are not directly implicated as another nuclear targeting signal. Apart from the PHD fingers, the C terminus has the proline-rich region between the zinc fingers and a 70-amino acid region distal to the second PHD finger (Fig. 1). We conclude that the C terminus alone is sufficient to transport AIRE into the nucleus. Taken together with the results presented above, this suggests that AIRE has two functional nuclear localization signals, the consensus NLS in the N terminus and another thus far undefined in the C terminus.

Full-length AIRE Protein Is Needed for the Formation of Nuclear Dot-like Staining—In addition to the tubular cytoplasmic localization, AIRE has been reported to exhibit a nuclear-staining pattern resembling PML nuclear bodies, also known as ND10, nuclear dots, or potential oncogenic domains (PODs) (7, 9, 10, 12, 13). We performed transfections with wild-type AIRE and several deletion constructs to determine the domain(s) needed to produce the nuclear dot-like structures.

Interestingly enough, none of the tested deletion constructs showed the dot-like nuclear staining (Table I). When nuclear localization was seen, the pattern was diffuse. This was also true for the patient mutation L28P and the double mutant L28P/K83E, both of which have diminished transcriptional activating activity (10% and 3% respectively) compared with wild-type AIRE (4). The APECED patient mutation K83E also failed to produce the nuclear dot staining, although it is fully functional in transcriptional activation (see below). On the other hand, the PHD zinc finger mutations C302P and C437P, which had no discernible effect on subcellular localization, caused severely decreased transcriptional activation (see below). As shown previously (4), the L28P and L28P/K83E mu-

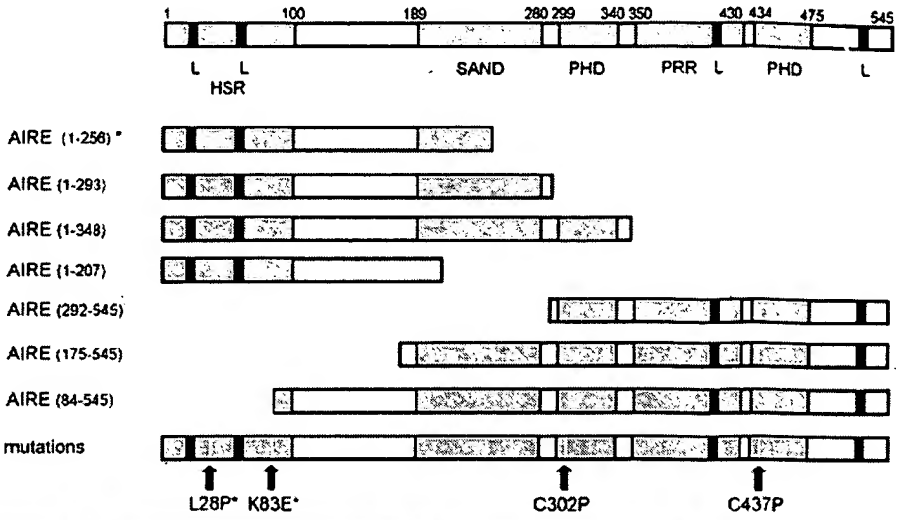

FIG. 1. **Schematic representation of the AIRE protein and its different deletion constructs.** *Mutations* indicate the sites of the missense mutations in the constructs AIRE L28P, AIRE L28P/K83E, AIRE K83E, AIRE C302P, AIRE C437P, and AIRE C302P/C437P. The *asterisk* denotes mutations found in APECED patients (R257*X*, L28P, and K83E).

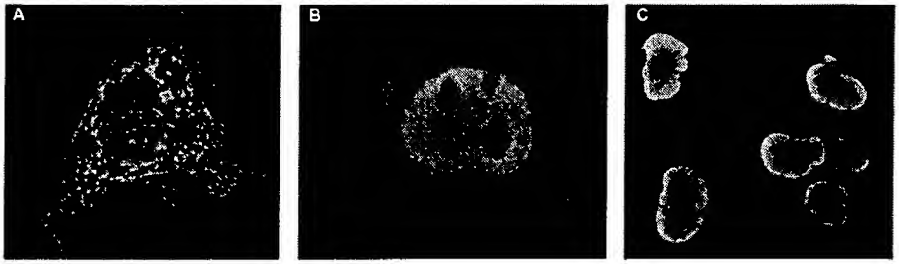

FIG. 2. **The tubular staining pattern of wild-type AIRE is abolished by the L28P patient mutation.** Immunofluorescence of COS-1 cells transiently transfected with either wild-type AIRE, AIRE L28P mutant, or AIRE-(84–545). In contrast to the microtubular staining of wild-type AIRE (*A*) the L28P mutant is located diffusely both in the cytoplasm and nucleus (*B*), indicating that the N terminus of AIRE is needed for the tubular localization. The AIRE-(84–545) construct (*C*) also lacks the tubular localization and moreover is almost exclusively located in the nucleus, indicating that a nuclear export signal resides in the N terminus of AIRE.

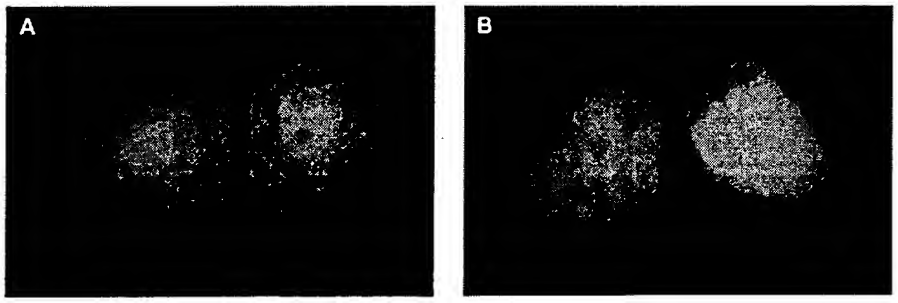

FIG. 3. **The AIRE consensus nuclear localization signal is functional.** Cloned downstream of GFP, a short fragment containing the nuclear localization signal (amino acids 101–141) confers nuclear localization to GFP (*B*), which normally is located both in the cytoplasm and nucleus (*A*). Nuclei were stained *blue* with DAPI.

tants were unable to homodimerize. We thus tested the C302P and C437P mutants for homodimerization by GST pull-down and found that both could form homodimers as efficiently as the wild-type protein (not shown).

Leptomycin B Leads to Increased Nuclear Localization of AIRE—Leptomycin B (LMB) is a specific inhibitor of CRM-1-mediated nuclear export (14–16), a process that has recently been shown to be important for the function of several proteins, including transcription factors (17). Different nuclear export signal sequences have been described, many of which are rich in leucine (18, 19). The presence of several putative export sequences on N- and C-terminal regions and the dual subcellular localization of AIRE in the cytoplasm and nucleus suggested that it might, in addition to being transported into the nucleus, also be actively exported from it. To test this hypothesis, cells transfected with AIRE were subjected to 5, 25, or 35 ng/ml LMB for 3 or 24 h. The proportion of cells exhibiting either a nuclear, cytoplasmic, or combined nuclear and cytoplasmic staining pattern was calculated. We found that treatment of cells with LMB at 15 ng/ml for 3 h increased the proportion of cells showing nuclear localization ~2-fold, whereas the proportion of cells with only cytoplasmic staining decreased accordingly (Fig. 4). Increasing the LMB concentration or treatment time had little effect on the outcome (not shown). To delineate the nuclear export signal we first tested N and C-terminal deletion constructs as putative signals were found in both termini. We found that AIRE-(1–207) showed a response to LMB indistinguishable from wild type, whereas the C-terminal constructs were unaffected (Fig. 4). In accordance, the AIRE-(84–545) deletion construct was localized almost exclusively in the nucleus (Fig. 2 and Table I). We suggest that AIRE is shuttled between the nucleus and cytoplasm, possibly to regulate its function.

AIRE Activates Transcription from the Interferon β Minimal Promoter—To assess the function of wild-type AIRE and the effect of patient mutations on that function, we devised an *in vivo* reporter assay. The reporter used contains the *IFNβ* minimal promoter (nucleotides −55 to +19) upstream of the luciferase gene. Wild-type or mutated AIRE was cotransfected with the appropriate reporter, and luciferase activity was measured 48 h post-transfection.

The reporter construct expressed small baseline amounts of

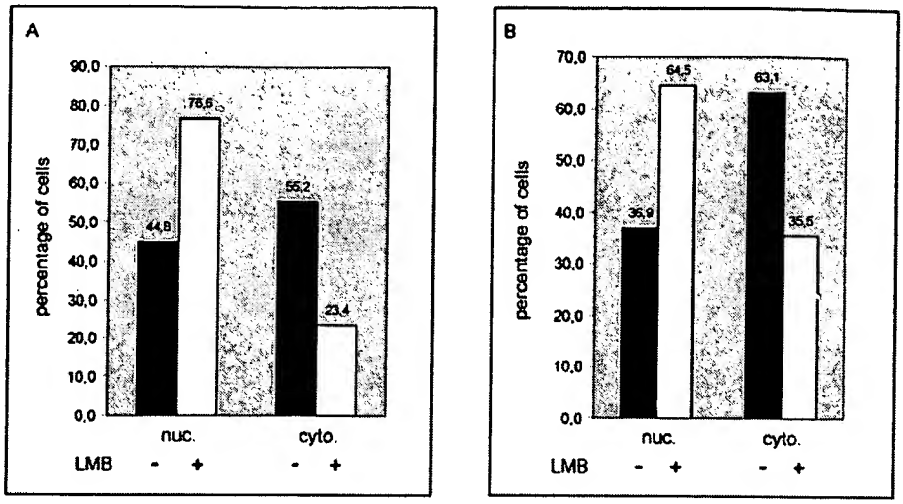

FIG. 4. **LMB treatment of AIRE-transfected cells leads to nuclear accumulation of AIRE.** Percentage of cells showing either nuclear (*nuc.*, cells showing only nuclear or nuclear and cytoplasmic staining) or cytoplasmic (*cyto.*, cells showing cytoplasmic staining only) localization are given. *A*, with wild-type AIRE the proportion of cells showing nuclear localization is approximately 2-fold after LMB treatment. *B*, AIRE-(1–207) shows a similar response to LMB suggesting that the N terminus harbors a nuclear export signal.

luciferase, and the addition of wild-type AIRE increased the transcription of the reporter gene ~5.5-fold (Fig. 5). To determine whether an activation domain could be demonstrated, we tested our deletion/mutation constructs (Fig. 1). The C-terminal constructs AIRE-(84–545) and AIRE-(292–545) showed an activation comparable with wild-type AIRE and the AIRE-(175–545) construct a slightly lower activation. In accordance, the constructs carrying mutations in the PHD fingers, AIRE C302P, AIRE C437P, and AIRE C302P/C437P, had reduced transcriptional activating capabilities. Thus, wild-type AIRE can activate the *IFNβ* minimal promoter, and the PHD fingers are directly implicated as the activation domain.

DISCUSSION

Microtubular Localization of AIRE Is Induced by the N-terminal HSR Domain—As indicated by the results of transfection experiments with the different AIRE deletion and mutation constructs, the domain responsible for the localization into the cytoskeleton is the N terminus (Table I). We narrowed the minimal domain down to the first 207 amino acids. The most likely candidate and the only intact domain in this sequence is the HSR domain, comprising amino acids 1–100 of AIRE.

The exact identity of the tubular fibers AIRE colocalizes with is not entirely clear. Rinderle *et al.* (10) have shown that AIRE colocalizes with vimentin and microtubules in transfected COS and primary fibroblast cells. They also observed that the N-terminal construct (amino acids 1–209) localized in microtubules in primary fibroblasts but did not do so in COS cells. The results of another group are in concordance with our findings as to the common Finnish APECED mutation R257*X*: they found it to be localized in filamentous structures in the cytoplasm (5) (Table I). They also showed colocalization of AIRE with vimentin (9).

We have shown earlier that the AIRE HSR domain has a predicted four-helix bundle structure and that it mediates AIRE-AIRE homodimerization (4). Interestingly, the L28P patient mutation not only abolishes homodimerization and transcriptional activation properties (4) but is also unable to localize in the microtubular structures of the cytoskeleton. This might indicate that homodimerization through the HSR domain is a prerequisite for the tubular localization.

All but two AIRE missense mutations found so far in APECED patients are in the HSR domain (1, 2, 5, 7), which indicates that it is sensitive to conformational changes (4) and likely to be important in the function of AIRE. Further evidence for the importance of the HSR domain is offered because we report here that it mediates the localization of AIRE to microtubular structures. It has been shown that the IκBα protein, a key molecular target in the regulation of NFκB activity, is localized in the microtubule network through its interaction with a cytoskeleton-associated protein (20). Although it is not yet known whether AIRE interacts with the microtubular network directly or via some other protein-protein interaction, we suggest that the deposition of AIRE in these structures plays an important part in the regulation of its function.

The Consensus Nuclear Localization Signal Is Functional—To address the functionality of the nuclear localization signal found in the N terminus of AIRE, we studied its ability to transport GFP into nuclei. A polypeptide containing the bipartite AIRE NLS (amino acids 101–141) effectively transported the GFP·NLS fusion into the nuclei of transiently transfected COS-1 cells.

The C Terminus of AIRE Contains a Nuclear Transport Signal—In studying the role of the PHD finger-containing C terminus in the subcellular localization of AIRE we rather surprisingly found that the C terminus alone can function as a mediator of nuclear transport irrespective of the consensus nuclear localization signal in the N terminus. The PHD zinc fingers, however, are not likely to be responsible for the nuclear transport as missense mutations (C302P, C437P, and C302P/C437P) fail to alter the subcellular localization of the protein. It can be hypothesized that AIRE, through the C terminus, binds some protein in the cytoplasm, thus inducing transport into the nucleus and subsequent transcriptional regulating activity. We conclude that the C terminus of AIRE is involved in its nuclear transport, possibly as a means of regulating its activity as well as subcellular distribution. Thus, AIRE has two functional nuclear localization signals, the consensus NLS of the N terminus and another as yet undefined in the C terminus.

AIRE Harbors a Nuclear Export Signal—The dual subcellular localization of AIRE and the presence of putative nuclear export signals prompted us to consider the possibility of nuclear export of AIRE. Indeed, we found that leptomycin B, an inhibitor of CRM-1-mediated nuclear export, increased ~2-fold the proportion of cells showing a nuclear localization whereas the number of cells showing only cytoplasmic distribution decreased accordingly (Fig. 4). The nuclear export signal was found to most likely reside in the N terminus of AIRE because AIRE-(1–207) showed a response to LMB indistinguishable from wild type. Further, AIRE-(84–545), lacking a putative nuclear export signal and the microtubular localization-mediating HSR domain was almost exclusively located in the nucleus. LMB treatment of AIRE-expressing cells did not lead to complete nuclear accumulation. In the case of AIRE, the effect

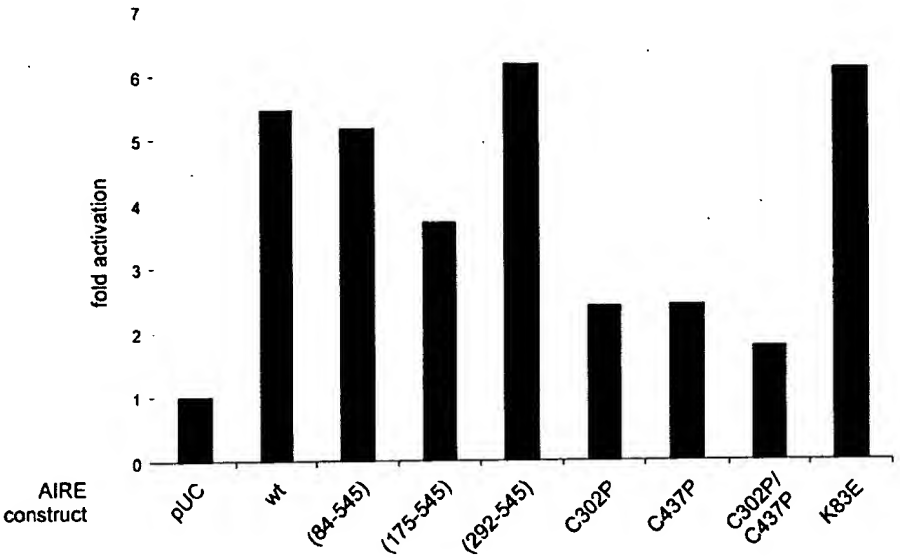

FIG. 5. **AIRE activates transcription from the interferon β minimal promoter.** Wild-type AIRE (*wt*) leads to an activation ~5.5-fold. The C-terminal constructs AIRE-(84–545) and AIRE-(292–545) show a similar activation whereas AIRE-(175–545) activates slightly less efficiently. The PHD zinc finger-disrupting mutations C302P and C437P lead to a severely decreased activation. Interestingly, the patient mutation K83E has no effect on the activation.

is seen as an increase in the number of cells showing nuclear staining. We suggest that this is partially because of the localization of AIRE in the microtubular network of the cytoplasm. Binding to these structures in the cytoplasm could inhibit nuclear transport to some extent. Thus, a tempting hypothesis is that AIRE is shuttled between the nucleus and cytoplasm to regulate its activity, the microtubular network functioning as a cytoplasmic storage compartment and the nuclear dots as the actual site of AIRE function.

AIRE Activates the IFNβ Minimal Promoter—To study the transcriptional regulating properties of AIRE, we devised a functional assay utilizing the *IFNβ* minimal promoter upstream of the luciferase gene. We found that wild-type AIRE activated the *IFNβ* minimal promoter 5.5-fold (Fig. 5). This finding is in agreement with previous studies where AIRE was found to activate transcription when fused to a heterologous DNA binding domain (4, 5). However, the functional significance of the activation requires further study. Furthermore, we showed that the AIRE-(292–545) construct, containing the PHD fingers and the proline-rich region inbetween, was also fully functional (Fig. 5). The AIRE-(84–545) construct, containing in addition the SAND domain, was equally active. We also saw activation by AIRE-(175–545), albeit slightly lower than wild type, which was absent in a GAL4-based reporter assay (4), probably indicating that the minimal promoter assay used here is the more sensitive. The PHD fingers of the C terminus are thus implicated as an activation domain.

To summarize, we show here that different domains of the AIRE protein serve distinct roles in its subcellular localization and function. The N terminus confers microtubular localization via the HSR domain. A nuclear export signal is also likely to reside in the same domain. As we have earlier shown that the HSR domain is required for the homodimerization of AIRE, its importance becomes evident. The N terminus of AIRE is also the domain most conserved in mouse Aire, exhibiting almost 100% homology (21, 22). The C terminus contains an additional nuclear localization signal, and the PHD zinc fingers function as an activation domain.

The present study adds to increasing evidence that define AIRE as a transcriptional regulator. The exact mechanism of how AIRE exerts transcriptional activation and influences its protein partners, though, remains unresolved. The manifesting symptoms of APECED indicate a defect in the mechanisms inducing tolerance to self-antigens. The lack of tolerance seen in APECED might be considered to arise from impaired negative selection of T-cells. Although the mechanisms of tolerance induction in the thymus medulla are also largely unknown, the importance of medullary epithelial cells and dendritic antigen-presenting cells has been established (23). In light of the limited expression of AIRE in these very cells and because it is a transcriptional regulator, we suggest that AIRE may regulate genes acting in negative selection in the thymus medulla. The data presented here give a firm basis for further study on the functional significance of the alternating subcellular localization of AIRE, and the specific mechanisms of its action. Especially, the recognition of the proteins AIRE is involved with in each subcellular location will help elucidate this. We believe that the resolution of the aforementioned issues as well as the results shown in this study will eventually give important information on the induction and maintenance of immune tolerance.

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REFERENCES

1. Nagamine, K., Peterson, P., Scott, H. S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K. J., Lalioti, M. D., Mullis, P. E., Antonarakis, S. E., Kawasaki, K., Asakawa, S., Ito, F., and Shimizu, N. (1997) *Nat. Genet.* **17,** 393–398
2. The Finnish-German APECED Consortium (1997) *Nat. Genet.* **17,** 399–403
3. Gibson, T. J., Ramu, C., Gemund, C., and Aasland, R. (1998) *Trends. Biochem. Sci.* **23,** 242–244
4. Pitkänen, J., Doucas, V., Sternsdorf, T., Nakajima, T., Aratani, S., Jensen, K., Will, H., Vähämurto, P., Ollila, J., Vihinen, M., Scott, H. S., Antonarakis, S. E., Kudoh, J., Shimizu, N., Krohn, K., and Peterson, P. (2000) *J. Biol. Chem.* **275,** 16802–16809
5. Björses, P., Halonen, M., Palvimo, J. J., Kolmer, M., Aaltonen, J., Ellonen, P., Perheentupa, J., Ulmanen, I., Peltonen, L. (2000) *Am. J. Hum. Genet.* **66,** 378–392
6. Peterson, P., Nagamine, K., Scott, H., Heino, M., Kudoh, J., Shimizu, N., Antonarakis, S. E., and Krohn, K. J. (1998) *Immunol. Today* **19,** 384–386
7. Heino, M., Peterson, P., Kudoh, J., Nagamine, K., Lagerstedt, A., Ovod, V., Ranki, A., Rantala, I., Nieminen, M., Tuukkanen, J., Scott, H. S., Antonarakis, S. E., Shimizu, N., and Krohn, K. (1999) *Biochem. Biophys. Res. Commun.* **257,** 821–825
8. Heino, M., Peterson, P., Sillanpaa, N., Guerin, S., Wu, L., Anderson, G., Scott, H. S., Antonarakis, S. E., Kudoh, J., Shimizu, N., Jenkinson, E. V., Naquet, P., and Krohn, K. J. (2000) *Eur. J. Immunol.* **30,** 1884–1893
9. Bjorses, P., Pelto-Huikko, M., Kaukonen, J., Aaltonen, J., Peltonen, L., and Ulmanen, I. (1999) *Hum. Mol. Genet.* **8,** 259–266
10. Rinderle, C., Christensen, H. M., Schweiger, S., Lehrach, H., and Yaspo, M. L. (1999) *Hum. Mol. Genet.* **8,** 277–290
11. Ausubel, F. M., Brent, R., Kingdom, R. E., Moore, D. M., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) *Current Protocols in Molecular Biology,* Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.
12. Sternsdorf, T., Grotzinger, T., Jensen, K., and Will, H. (1997) *Immunobiology* **198,** 307–331
13. Doucas, V., and Evans, R. M.(1996) *Biochim. Biophys. Acta* **1288,** 25–29
14. Fukuda, M., Asano, S., Nakamura, T., Adachi, M., Yoshida, M., Yanagida, M., and Nishida, E. (1997) *Nature* **390,** 308–311

15. Kudo, N., Matsumori, N., Taoka, H., Fujiwara, D., Schreiner, E. P., Wolff, B., Yoshida, M., and Horinouchi, S. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 9112–9117
16. Nishi, K., Yoshida, M., Fujiwara, D., Nishikawa, M., Horinouchi, S., and Beppu, T. (1994) *J. Biol. Chem.* **269**, 6320–6324
17. Komeili, A., and O'Shea, E. K. (2000) *Curr. Opin. Cell Biol.* **12**, 355–360
18. Adam, S. A. (1999) *Curr. Opin. Cell Biol.* **11**, 402–406
19. Nakielny, S., and Dreyfuss, G. (1997) *Curr. Opin. Cell Biol.* **9**, 420–429
20. Crepieux, P. K. H., Leclerc, N., Spencer, W., Richard, S., Lin, R., and Hiscott, J. (1997) *Mol. Cell. Biol.* **17**, 7375–7385
21. Mittaz, L., Rossier, C., Heino, M., Peterson, P., Krohn, K. J., Gos, A., Morris, M. A., Kudoh, J., Shimizu, N., Antonarakis, S. E., and Scott, H. S. (1999) *Biochem. Biophys. Res. Commun.* **255**, 483–490
22. Shi, J. D., Wang, C. Y., Marron, M. P., Ruan, Q. G., Huang, Y. Q., Detter, J. C., and She, J. X. (1999) *Autoimmunity* **31**, 47–53
23. Klein, L., and Kyewski, B. (2000) *Curr. Opin. Immunol.* **12**, 179–186